Forensic Science International: Genetics Supplement Series 2 (2009) 294–296

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

Research article

FishPopTrace—Developing SNP-based population genetic assignment methods to investigate illegal fishing

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ARTICLE INFO

Article history:
Received 14 August 2009
Accepted 22 August 2009

Keywords:
IUU fishing
Traceability
Origin assignment
Population genetics
SNP markers
Forensics

ABSTRACT

The FAO estimates that 80% of marine fish stocks are fully or overexploited worldwide. Illegal unreported and unregulated (IUU) fishing contributes vastly to this condition, and poses a severe threat to marine ecosystems. Controlling for compliance and enforcing fishing regulations is hampered by difficulties in identifying the geographic origin of fish and fish products, at point of landing and further down the food supply chain. While forensic genetic species identification methods are routinely employed to investigate commercial fraud, there are at present no validated methods for identifying the geographic origin of marine fish.

FishPopTrace is an international project, funded by the EU framework programme (FP7), aiming to generate forensically validated panels of SNP markers for geographic origin assignment in four commercially important fish species, cod (*Gadus morhua*), hake (*Merluccius merluccius*), herring (*Clupea harengus*) and common sole (*Solea solea*). 454-sequencing with sample tagging is employed to generate large numbers of population informative candidate SNP loci in each species. Selected SNPs are subsequently genotyped using Illumina 1536-arrays across populations to provide high resolution maps of genetic variation. Panels comprising subsets of these markers will ultimately be validated for traceability and enforcement applications.

1. Introduction

Monitoring, control and surveillance (MCS) are key to fisheries management schemes, and the availability of efficient law enforcement measures is indispensible for their successful implementation. The need for effective MCS strategies is highlighted by the global problem of illegal, unreported and unregulated (IUU) fishing, estimated to be worth €10–20 billion annually [1]. Moreover, there are numerous reported cases where fish product has been sold using false labelling, showing that global criminal conduct extends deeply into the fisheries supply chain [2].

Forensic genetic analysis has great potential for the investigation of illegal fishing and food fraud. Genetic species identification from fish and fish products based on comparative mitochondrial DNA gene sequences is well established and a number of prominent examples of its successful application to legal prosecution exist [3]. However, existing fisheries legislation often refers to fish stocks or geographic regions as relevant units for law enforcement and this imposes the need for methods identifying the population of origin of landed fish.

The development of methods for assigning an individual to its genetic population of origin has been the focus of continued research over the past decade [4] and more recently applied within the field of forensic genetics [5]. Multiple polymorphic markers applied across genetically divergent populations often enable a sample to be unambiguously assigned to a single source, while being excluded from all other candidate regions. This has been demonstrated for anadromous fish [6], however genetic divergence is often reduced in marine fishes which typically exhibit considerable geneflow among populations, requiring a large number of neutral markers or the inclusion of markers under selection to local environment to distinguish among regions with sufficient power.

This paper describes the work undertaken within FishPopTrace, a project funded under the European Union FP7 Programme, which aims to generate a series of forensically validated marker panels for the identification of four commercially important fish species, cod (*Gadus morhua*), hake (*Merluccius merluccius*), herring (*Clupea harengus*) and common sole (*Solea solea*) [7]. Specifically we introduce the research and development pipeline (Fig. 1a) describing the methods employed for the large scale discovery,

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doi:10.1016/j.fsigss.2009.08.108

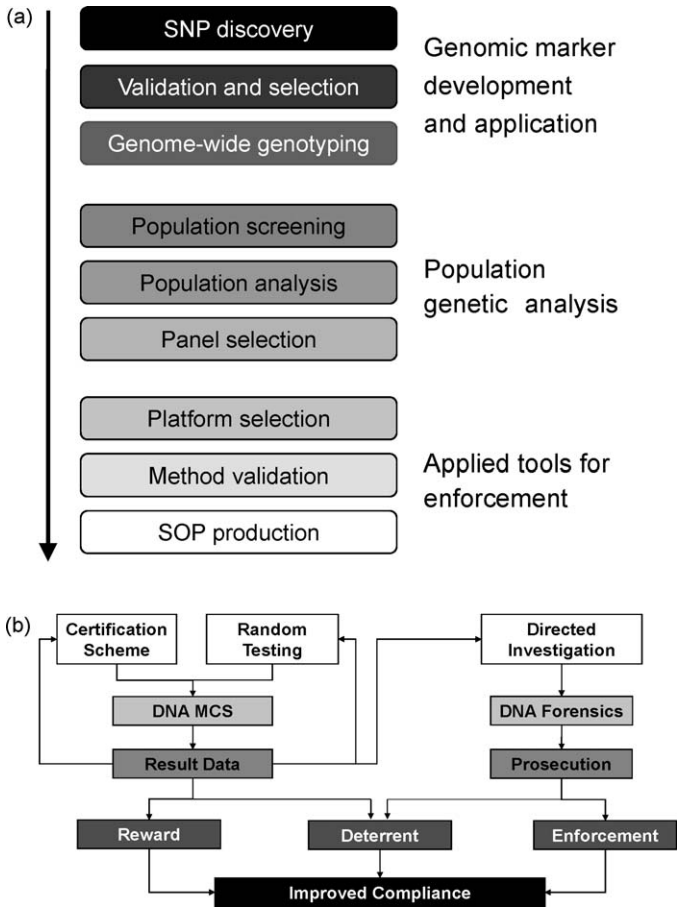

Fig. 1. (a) The principal phases of research and development in the FishPopTrace project to establish population informative genetic markers in support of fisheries control and enforcement and (b) flow diagram depicting the impact of genetic identification methods on monitoring (DNA MCS) and enforcement (DNA forensics) to improve compliance with fishing regulations.

selection and genotyping of SNP markers in each species and explain how they are to be subsequently validated as forensic tools.

2. Methods

Sampling: Target species specimens were collected from the Eastern Mediterranean, along the Atlantic coastline of the Iberian Peninsula, the Bay of Biscay, North Sea, Baltic Sea up to the Barents Sea. The exact sampling locations are visualized on a GIS map under https://fishpoptrace.jrc.ec.europa.eu/web/fishpoptrace/sampling/gis.

SNP discovery: For each species, RNA samples were collected from geographically distinct individuals. cDNA libraries were produced and cDNA from individual fish was tagged before being pooled and analysed on a 454-sequencer. The resulting sequence data was assembled into contigs, aligned and analysed for the presence of SNP markers using a number of filtering and quality control criteria.

Genotyping: Up to 1536 SNPs per species have been selected for inclusion in an Illumina GoldenGate® genotyping assay. By the end of 2009, these will have been assessed in around 1000 individuals across the whole geographic range covered by the sampling scheme. This will enable fine scale population structure to be derived and the individual SNPs identified that contribute most to geographic population differentiation. The most powerful markers will be used to form SNP panels for each species that are capable of unambiguously assigning an individual to its geographic origin.

Forensic validation: Developmental validation of all markers employed in the final panels, together with inter-laboratory calibration between the consortium partners will be carried out in accordance with SWGDAM guidelines where possible. The final genotyping platform has yet to be selected and will be dependent on the number of markers required. Forensic protocols for population identification including appropriate statistical methods for data analysis will be produced. The resulting forensic assays will be integrated into an accredited (ISO17025) and transferable forensic framework.

Complementary technologies: Following a holistic approach the FishPopTrace consortium is also exploring the potential and applicability of otolith microchemistry, fatty acid analysis, proteomics, gene expression and microarrays as additional tools for fisheries control and enforcement.

Supporting database: In addition to the existing FishPopTrace sampling database (https://fishpoptrace.jrc.ec.europa.eu/sampling) a genetic database will be created supporting data acquisition and mining for the analysis of fish populations and traceability. In line with the commitment of FishPopTrace to maximally engage with stakeholders and end-users, the database will provide public access to selected data as well as statistical tools for data analysis.

3. Results

Over 100 million bases of sequence data have been generated to date, clustering into between 2000 and 7000 contigs per species. From this data we have discovered approximately 7500 candidate SNP markers per species which are currently being validated *in silico* prior to inclusion on the 1536 SNP chip. All population samples have been collected and a validation study of sample type and sample treatment is ongoing. The project is scheduled to be completed by March 2011.

4. Discussion and conclusion

The results to date are encouraging. Large numbers of candidate SNPs have been discovered and we are optimistic that there should be a sufficient pool of markers available to resolve population structure in each species. Although less variable than STR markers, SNPs have been selected here for their ease of inter-laboratory calibration and to allow for the possibility of developing informative markers that are under selection for environmental geographic variables.

The outputs of FishPopTrace will provide forensic DNA technology to the fishing industry and control authorities to facilitate MCS (Fig. 1b). Inclusion of genetic identification in certification schemes or random testing should help deter illegal practices and enhance confidence in product authenticity, rewarding legal fishing practices and thereby improving compliance. In tandem, intelligence-led investigations can utilize genetic identification in a forensic context to prosecute offenders, resulting in direct enforcement and associated deterrent effects. It is hoped that in this way, the project will contribute to a reduction in IUU fishing and conservation of remaining marine resources.

Conflict of interest

None.

Role of funding statement

FishPopTrace is funded by the European Community's Seventh Framework Programme under grant agreement no. KBBE-212399 in the area of "Food, Agriculture and Fisheries, and Biotechnology".

The sponsor had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; nor in the decision to submit the paper for publication.

Acknowledgments

FishPopTrace is funded by the European Community's Seventh Framework Programme. We are grateful to the Canadian Cod Genome Project for providing access to their cod SNP panel.

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